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Market Quality and Rate of Respiration of HEAD LETTUCE Held in Low-Oxygen Atmospheres

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Market Quality and Rate of Respiration of Head Lettuce Held in Low-Oxygen Atmospheres

By W. J. LIPTON, *plant physiologist*,[1] *Market Quality Research Division, Agricultural Research Service, U.S. Department of Agriculture*

SUMMARY

The market quality of head lettuce was evaluated after 7 days at 36°, 41°, or 50° F. in atmospheres containing 0, ¼, ½, 1, 2, 5, or 8 percent O_2 or in air and after an additional 3 or 4 days in air at 50° F.

Concentrations of 0 and ¼ percent O_2 injured the lettuce. Gray dead patches on the wrapper and cap leaves and dead young heart leaves were the principal symptoms of injury. The injuries became more severe as the temperature was increased.

Russet spotting was reduced substantially in all low-oxygen (O_2) atmospheres tested, regardless of temperature. The incidence of pink rib was not affected by low-O_2 atmospheres. The severity, however, was reduced in ½ percent O_2 when all test temperatures are considered, but increased in 2 percent O_2 at 50° F.

Decay was reduced, but not significantly, in 1 percent O_2. Low temperature was more effective in reducing decay than low O_2.

Butt discoloration was generally less intense in ½ and 1 percent O_2 than in air, but not after 3 or 4 additional days in air.

The general appearance of nontrimmed lettuce was not affected significantly by holding it in ½ to 8 percent O_2 at 36° or 41° F.; although lettuce held at 50° in 1, 2, 5, or 8 percent O_2 for 7 days plus 3 or 4 days in air appeared significantly greener and fresher than that held in air throughout. However, the quality of lettuce held in air at 36° or 41° was equal to and often superior to that held under low O_2 at 50°. After the lettuce was trimmed, the only benefit from low O_2 was a significant reduction in russet spotting.

The rate of respiration (carbon dioxide [CO_2] production) was reduced by about a third to a half in ½, 1, or 5 percent O_2 compared with that in air, depending upon O_2 concentration and temperature. At 41°, a 50-percent decrease in the rate of CO_2 production would reduce vital heat production from 40,000 to 20,000 B.t.u. per day per 20-ton load of lettuce. This reduction of 20,000 B.t.u. is equivalent to the refrigeration capacity of about 140 pounds of ice or 115 pounds of liquid nitrogen (N_2).

When N_2 is used as the refrigerant or as a supplement to mechanical refrigeration, the O_2 concentration should be at least 1 percent, never less than ½ percent. For maximum quality retention, low temperatures must accompany low-O_2 atmospheres.

BACKGROUND

Liquid N_2 has been used recently as a refrigerant or as a supplement to mechanical refrigeration in some shipments of western lettuce. The liquid N_2 expands into a gas as it enters the load compartment, displaces the air, and thereby lowers the O_2 content. Parsons and others (*6*)[2] found that very low O_2 concentrations reduced russet spotting of lettuce, but Watada and others (*12*) reported that O_2 concentrations from 1 to 15 percent had little effect on the rate of deterioration of lettuce. Further, Stewart

[1] Fresno, Calif.

[2] Italic numbers in parentheses refer to Literature Cited, p. 9.

(*10*) noted undesirably high transit temperatures in shipments of lettuce refrigerated by N_2. Harvey (*3*) cited evidence that atmospheres low in O_2 injured some crops, although Parsons and others observed no injury on lettuce held in very low O_2 concentrations for 10 days at 33° F.

These divergent results needed to be clarified so that sound recommendations could be made to the lettuce industry on the safety or usefulness of low-O_2 atmospheres. Consequently, tests were conducted to evaluate the response of lettuce to such atmospheres at various temperatures.

Since a reduction in the heat of respiration is claimed as one advantage of shipping lettuce under low O_2 (*1*), respiration was determined under various conditions.

METHODS AND MATERIALS

Sources of test lettuce: —Lettuce of the Great Lakes type was obtained in 1964 and 1965 from the Salinas Valley or near Mendota in the San Joaquin Valley of California. The lettuce was cut and packed by field crews, but, to reduce injury, only 18 heads were placed in each carton, instead of the customary 24. Lettuce from the Salinas Valley was vacuum cooled less than 1 hour after harvest and transported under refrigeration to the U.S. Department of Agriculture laboratory at Fresno, Calif.; that from the San Joaquin Valley was not vacuum cooled prior to storage because of the short transit time involved.

Atmospheres and temperatures during holding: —The lettuce was placed in stainless steel chambers and exposed to the appropriate atmospheres the same day it was harvested, or within 24 hours. The lots for each treatment contained an equal number of heads from each original carton for a total of 16 or 18. Wet towels maintained a high relative humidity in the chambers. Adjacent heads were from different cartons to avoid introduction of potential nests of decay.

After closure, some chambers were flushed with N_2 until the desired O_2 concentration was nearly reached. The atmospheres were maintained by a continuous flow of the appropriate mixtures of O_2 and N_2 dispensed from high-pressure cylinders. Atmospheres in chambers used as controls contained 21 percent O_2 (air) and were maintained by a continuous flow of air.

The desired O_2 concentration was generally reached within 4 to 6 hours after closing the chambers. The lettuce was held in O_2 concentrations of zero, ¼, ½, 1, 2, 5, 8, and 21 percent and at temperatures of 36°, 41°, or 50° F. Zero and ¼ percent O_2 were not tested at 50° because these atmospheres seriously injured the lettuce at 36° and 41°. The N_2 used to obtain zero percent O_2 may have contained undetected traces of O_2, which, however, were not sufficient to prevent low-O_2 injury. The rate of flow was sufficient to keep CO_2 levels below ½ percent. The effluent gas had nearly the same O_2 concentration as that entering the chambers, as monitored with a Burrell[3] gas analyzer.

Evaluation of market quality: —The effect of each O_2-temperature treatment on lettuce was evaluated in at least three tests. Half the lettuce was examined after 7 days in the atmospheres evaluated (simulated transit period), and the remainder after an additional 3 or 4 days in air at 50° F. (simulated wholesale-retail period). Each head was rated for russet spotting, pink rib, and decay on a scale from 1 to 9 (1=defects absent, 3=trace, 5=slight, 7=moderate, 9=severe). Observations on butt discoloration and injury from low-O_2 concentration were made when appropriate. The general appearance of the heads with and without wrapper leaves was rated on a scale from 9 to 1 (9=field fresh, no defects; 7=good, minor defects; 5=fair, objectionable defects that can be removed before sale; 3=poor, generally unsalable; 1=inedible).

The large number of treatments precluded their incorporation in any one test. Thirteen

[3] Trade names and the names of commercial companies are used in this publication solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

tests were conducted during the two seasons. Each test included eight treatments, three low-O_2 concentrations and one air control at each of two temperatures. A given treatment was tested at intervals during a growing season to avoid seasonal bias. The combined data from all tests were evaluated on an equal basis by applying the analysis of variance (Duncan's Multiple Range Test) for unequal sample size to the data which were calculated as a percentage of averages obtained for the lots held in air for a given temperature. (For example, average severity rating for pink rib at 50° F. in air, 4.0; in 2 percent O_2, 6.0; relative value for air, 100; for 2 percent O_2, 150.) The results of tests that differed substantially from the trend were also analyzed separately to gain information that would be lost in the averages of all tests.

Determination of respiration rates: —CO_2 production was measured on lettuce grown in the Imperial Valley of California (January to March 1966) and obtained from a local wholesaler 24 to 36 hours after harvest. The lettuce was held in glass jars at 41° or 50° F. in ½, 1, 5, or 21 percent O_2 (air). Six heads were used for the 41° treatments, five heads for the 50° treatments. Each treatment was tested twice. The graphs in figure 1 represent the average values of the replicates at each temperature.

CO_2 was determined with a positive nondispersive-type infrared analyzer (Liston-Becker, model 15A) connected to a Varian strip chart recorder set at zero to 0.250 percent CO_2 full scale. A given gas sample passed through the analyzer for about 4 minutes at about 1½-hour intervals, as regulated by an automatic manifold. CO_2-free air and O_2-N_2 mixtures were used in the respiration studies.

RESULTS

Effect of Low-Oxygen Atmospheres on Market Quality

Injurious effects of low oxygen: —Extensive gray, dead patches developed on the wrapper leaves of lettuce held 7 days in zero percent O_2 at 36° or 41° F. These injuries were present to a lesser degree on wrapper leaves held in ¼ percent O_2 (pl. 1), but were more widespread and severe after the additional 3 or 4 days in air at 50° than at the first examination. Cap leaves of heads held previously in zero percent O_2 also developed gray, dead patches. Injury to heart leaves from low-O_2 concentrations appeared as reddish-brown spots (pl. 2) on the midribs of leaves near the center of the head. Zero percent O_2 caused injury to wrapper, cap, and heart leaves, but ¼ percent O_2 caused the death of only the heart leaves.

Both the incidence and severity of the external and internal injuries increased as the temperatures increased. In one test at 50° F., the heart leaves died even in 0.3 to 0.4 percent O_2. The injuries developed further after removal of the lettuce to air at 50°, and decay often affected the injured wrapper leaves during this simulated wholesale-retail period.

Off-flavors—sweet, flat, not typical of lettuce —were detected in heads held in zero and ¼ percent O_2 and were sometimes more prominent in samples held at the higher concentration. These off-flavors generally disappeared after the lettuce was held 3 or 4 days in air at 50°.

General effects on quality:—Substantial inhibition of russet spotting (*4*) was the principal benefit of low-O_2 atmospheres in tests where this disorder occurred. The incidence was reduced about equally in zero to 8 percent O_2 and at all three temperatures (table 1).

Not only the incidence, but also the severity of russet spotting was reduced in the low-O_2 atmospheres. The severity, as a percentage of that in air, ranged from 29 to 66 percent, both values being significantly lower (99-percent probability level) than that in air. Differences among individual treatments were not tested statistically because the sporadic occurrence of russet spotting resulted in too few data for reliable analysis.

The incidence of pink rib, which was evident in all tests and in all treatments, was not affected significantly by low-O_2 atmospheres. The incidence, as a percentage of that in air, ranged from 74 percent in ½ percent O_2 to 103 percent in 8 percent O_2.

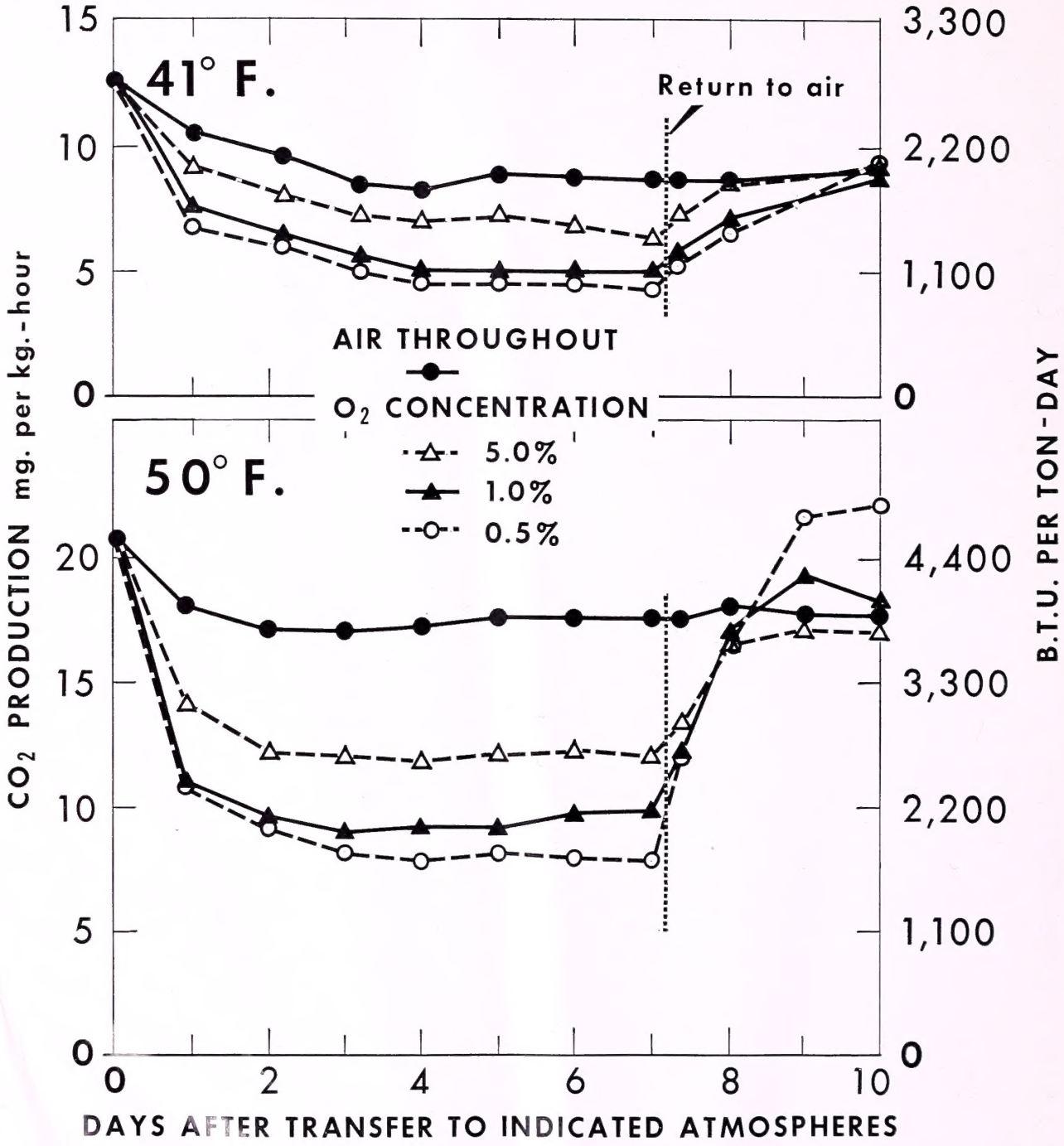

FIGURE 1.—CO_2 production by lettuce held in various O_2 concentrations at 41° or 50° F. and after the return to air.

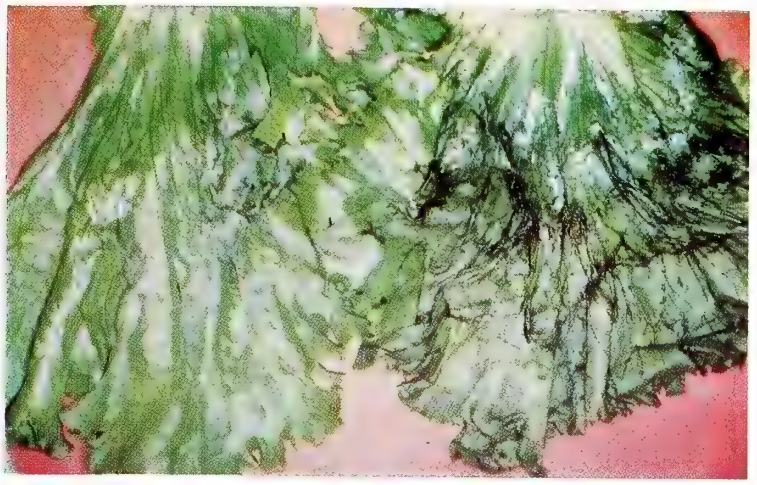

PLATE 1

Wrapper leaves from lettuce held 7½ days at 41° F. in ¼ (left) or 0 percent O_2 (right). Injured areas are dead and gray.

PLATE 2

Cross section through lettuce heads held 7½ days in 0 percent O_2 at 36° (left) or 41° (right) plus 4 days in air at 50°.

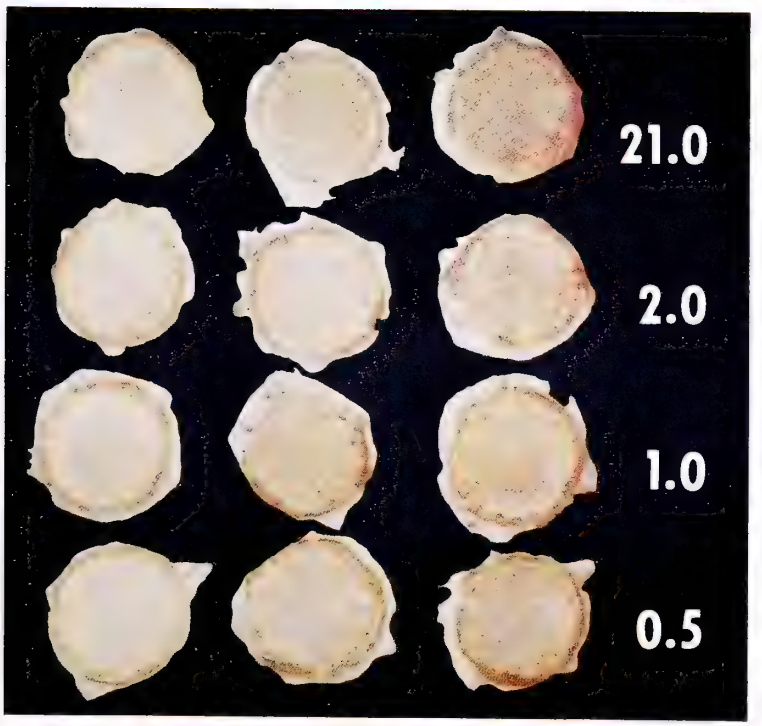

PLATE 3

Color of butts of lettuce held 7 days at 36° in 21, 2, 1, or ½ percent O_2. Note slightly lesser degree of discoloration after holding lettuce in ½ or 1 percent O_2; also, great variation among butts held in same atmosphere.

TABLE 1.—*Incidence of russet spotting, severity of pink rib, and overall quality of lettuce held in various low-oxygen atmospheres*

[All values expressed are relative to their percentages in air (21 percent O_2). Figures on a given line without a common letter differ at the 95-percent probability level. Averages are for all samples at any one O_2 concentration; they are not equal to the averages of each column because of the unequal numbers of samples in each treatment. At least 48 heads were evaluated for each treatment]

Storage temperature (° F.)	Values for lettuce held 7 days in the following percentages of oxygen [1] plus 3 or 4 days in air at 50° F.					
	½	1	2	5	8	21
Relative incidence [2] of russet spotting at —	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
36	3 a	21 a	7 a	13 a	19 a	100 b
41	0 a	17 a	19 a	0 a	13 a	100 b
50	([3])	0 a	0 a	0 a	0 a	100 b
Average	2 a	17 a	9 a	6 a	12 a	100 b
Relative severity [4] of pink rib at —						
36	84 a	83 a	89 a	99 a	98 a	100 a
41	81 a	85 a	82 a	98 a	98 a	100 a
50	80 a	127 cd	133 d	117 bcd	121 cd	100 abc
Average	82 a	96 ab	100 b	104 b	105 b	100 b
Relative rating [5] of overall quality at —						
36	103 a	104 a	103 a	107 a	106 a	100 a
41	103 a	105 a	104 a	106 a	101 a	100 a
50	120 b	138 b	129 b	123 b	127 b	100 a
Average	107 a	114 a	111 a	112 a	111 a	100 a

[1] Data for zero percent and ¼ percent O_2 not included because these atmospheres seriously injured the lettuce.
[2] Actual incidence in air averaged 33 percent at 36° F., 36 percent at 41°, and 14 percent at 50°; average for all temperatures was 31 percent.
[3] No russet spotting occurred in tests that included this treatment.
[4] Actual severity rating in air averaged 2.5 at 36° F., 3.3 at 41°, and 4.3 at 50°; average rating for all temperatures was 3.3. Rating scale used: 1 = none, 3 = trace, 5 = slight, 7 = moderate, 9 = severe.
[5] Actual quality rating of nontrimmed lettuce in air averaged 6.4 at 36° F., 6.2 at 41°, and 4.6 at 50°; average rating for all temperatures was 5.8. Rating scale used: 9 = excellent, 7 = good, 5 = fair, 3 = poor.

The severity of pink rib was reduced significantly in ½ percent O_2 (table 1). In contrast, the severity was intensified by 1, 2, 5, or 8 percent O_2 at 50° F. in almost every test; the increase was greatest in 2 percent O_2 (table 1). Pink rib was sufficiently severe to detract from the market value of much of the lettuce (see table 1, footnote 4). At 36° or 41°, none of the atmospheres affected the severity of pink rib significantly.

The effect of low-O_2 atmospheres on the incidence of decay was inconsistent. Although lettuce held in 1 percent O_2 had slightly less decay after the simulated transit period plus 3 or 4 days in air (the incidence was 66 percent of that in air) than that from the other atmospheres, none of the differences were statistically significant. Temperature exerted a greater influence on decay than O_2 concentration; the incidence of decay increased as temperature increased from 36° to 50° F. Heads held under low O_2 at 50° had consistently more decay than those held in air at 36° or 41°. No trend existed in respect to severity of decay. The decay that did occur was usually confined to the wrapper leaves and was not serious.

Butt discoloration varied widely within a given treatment but was generally less severe in ½ percent or 1 percent O_2 than in air (pl. 3). Holding the lettuce 3 or 4 days in air at 50° F. completely negated any differences that existed immediately after the lettuce was removed from ½ or 1 percent O_2. The cut surface remained white only at zero or ¼ percent O_2, concentrations that injured the lettuce severely.

The appearance of nontrimmed lettuce was affected very little by storage in ½ to 8 percent O_2 at 36° or 41° F., temperatures near and slightly above desirable levels. At 41°, lettuce held in low-O_2 (1 percent) concentrations was significantly superior to that held in air in only one test. However, atmospheres in the range of 1 to 8 percent O_2 improved the appearance of lettuce held at 50° F. (table 1). Lettuce in these treatments remained greener and had a fresher appearance than that held in air. This difference was not evident immediately after removal from low O_2, but only after the simulated wholesale-retail period in air at 50°. The appearance of lettuce held in air at low temperatures was commonly superior to that of lettuce held in low-O_2 concentrations at the next higher temperature. Therefore, low O_2 cannot be considered an improvement over low temperature in maintaining the market quality of lettuce when russet spotting is not a problem.

Trimming off the wrapper leaves removed all evidence of the favorable effect of low O_2 on general appearance. The amount of O_2 had little effect on trimmed lettuce either "on arrival" or after the simulated wholesale-retail period in air; only those benefits related to reduction in russet spotting remained. The effect of low O_2 on pink rib was variable, as previously noted.

Effect of Low-Oxygen Atmospheres on Respiration

Atmospheres with ½, 1, or 5 percent O_2 reduced the rate of CO_2 production of lettuce held at 41° or 50° F. (fig. 1), and the percentage reductions were similar at both temperatures. In ½ and 1 percent O_2 the rate of CO_2 production was reduced by about half of that in air and in 5 percent O_2 by about a third. These results parallel those for spinach (*7*) and for a variety of other plants (*2, 11*). As would be expected, the rates were higher at 50° than at 41°; in fact, at 50°, CO_2 production in ½ percent or 1 percent was about the same as at 41° in air.

The rate of CO_2 production adjusted to the lowered O_2 concentrations about 3 days after the change from air at both temperatures. In contrast, the return to the normal rate of CO_2 production after the lettuce was again placed in air was more rapid at 50° F. than at 41°. Further, at 50°, the rates for the lettuce held previously in ½ or 1 percent O_2 exceeded the rates of lettuce held in air throughout by 25 and 11 percent, respectively, before they leveled off—2 days after the return to air. Bourne and Ranson (*2*) observed a similar increase in CO_2 production after removal of rhododendron leaves from an N_2 atmosphere into air. This increase in CO_2 production above that existing in the air control, was almost absent at 41°.

DISCUSSION AND CONCLUSIONS

The foregoing results indicate that some definite, but limited, benefits may be derived from the use of certain low-O_2 atmospheres under conditions simulating the transcontinental shipment of head lettuce. The substantial reduction in russet spotting, even at O_2 concentrations as high as 8 percent, is clearly the major benefit. This response, previously observed by Parsons and others (*6*) in lettuce held at very low O_2 concentrations, is surprising at the higher O_2 concentrations because the rate of CO_2 production, a measure of metabolic activity, was substantially higher in 5 percent O_2 than in ½ or 1 percent O_2 (fig. 1). It appears, therefore, that russet spotting is inhibited at O_2 concentrations substantially higher than those that reduce CO_2 production appreciably. In contrast, the browning reaction associated with butt discoloration was prevented only in near anaerobic conditions, both in these tests and in those of Parsons and others. Pink rib, also the result of an oxidative discoloration, was actually enhanced in 1 or 2 percent O_2, but reduced in ½ percent O_2 at 50° F. These apparently paradoxical results cannot be clarified on the basis of the present tests, but they suggest that the circumstances that lead to russet spotting differ substantially from those that cause pink rib.

The small young heart leaves were most sensitive to low-O_2 injury and, therefore, would be a good indicator of the adequacy of the O_2 sup-

ply for lettuce during transit. The grayish-brown discoloration, especially at the leaf margins, was quite distinct from the reddish-brown discoloration caused by excess CO_2 (*12*). However, the reddish spots on the midrib of the central head leaves found in zero percent O_2 did resemble those described for CO_2 injury. Some of the external and internal injuries observed in lettuce held in zero percent and ½ percent O_2 resembled those attributed to "suboxidation" by Nelson (*5*). Parsons and others (*6*) did not observe these injuries, possibly because metabolic activity is lower at 33° than at 36° F. or above, or because not all entrapped O_2 was removed by flushing the chambers with N_2 for 10 to 15 minutes twice daily.

The lack of substantial effect of low O_2 on the general appearance of lettuce, especially after trimming, agrees closely with the results of Watada and others (*12*), who found that O_2 concentrations from 1 to 15 percent neither improved nor harmed lettuce held at 36° or 41° F. Watada and co-workers did not include 50° in their tests; this temperature, while undesirably high, does occur in lettuce shipped under liquid N_2 refrigeration (*10*). The retardation in quality loss resulting from 1 to 8 percent O_2 at 50°, but not at 41° or 36°, is related to the rate of deterioration at these temperatures. At 41° or below, sound lettuce remains in fairly good condition for nearly 2 weeks (*8*), so that color retention by wrapper leaves is not affected by low O_2 concentrations during shorter intervals. In contrast, at 50° the senescence retarding effect of low O_2 becomes noticeable because of the substantially higher rate of deterioration at this temperature. However, during long periods of storage, such as may occur in the supply of navy ships, the benefits of low O_2 also may be apparent near 40° F. Similarly, any beneficial effects of low O_2 on lettuce quality would be progressively less noticeable as the transit period is shortened.

The low incidence and severity of decay found in these laboratory tests, especially at 50° F., may be explained by the careful handling the lettuce received. In commercially packed and shipped lettuce, decay was substantial in O_2 concentrations of 2 percent or lower when transit temperatures were about 50° F. (*10*).

The data on CO_2 production allow determination of possible savings in the cost of refrigeration if lettuce is shipped in low-O_2 atmospheres. With a rate of heat production of about 2,000 B.t.u. per ton-day in air at 41° F., a 50-percent reduction lowers the heat of respiration from 40,000 to 20,000 B.t.u. per day in a 20-ton load of lettuce. This reduction of 20,000 B.t.u. is equivalent to the refrigeration capacity of about 140 pounds of ice or 115 pounds of liquid N_2. This relatively small saving lasts only as long as the O_2 level remains near 1 percent; if it rises, as has occurred when the supply of liquid N_2 was exhausted before arrival (*10*), normal rates of CO_2 production are approached rapidly or even exceeded. The savings would be slightly greater with lettuce respiring at the somewhat higher rates reported for lettuce from the Salinas Valley (*8*) or the Rio Grande Valley (*9*).

It follows from the above discussion that low-O_2 atmospheres do not benefit lettuce substantially except in respect to russet spotting, which was reduced significantly and about equally in ½ to 8 percent O_2. Further, for maximum quality retention, low temperatures (not above 41°) must accompany low-O_2 concentrations. When N_2 is used as a refrigerant or as a supplement to mechanical refrigeration, the O_2 level should remain near 1 percent or higher, never below ½ percent because of the danger of serious injury at lower concentrations.

These deterioration and respiration studies clearly show that low-O_2 atmospheres neither stop deterioration of lettuce nor "immediately put [lettuce] to sleep" as has been claimed (*1*).

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